

The Search for Resistance to EAB: Can Breeding be used to Develop EAB-Resistant North American Ash Species?

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Concept of Introgression

(transfer of a desired trait through breeding)

- Asian Ash species coevolved with EAB and are likely to have some level of resistance.

F. Mandshurica tested in common garden study in Novi, MI and demonstrates resistance. Rebek et al., 2008 Env. Entomol. 37:242-246

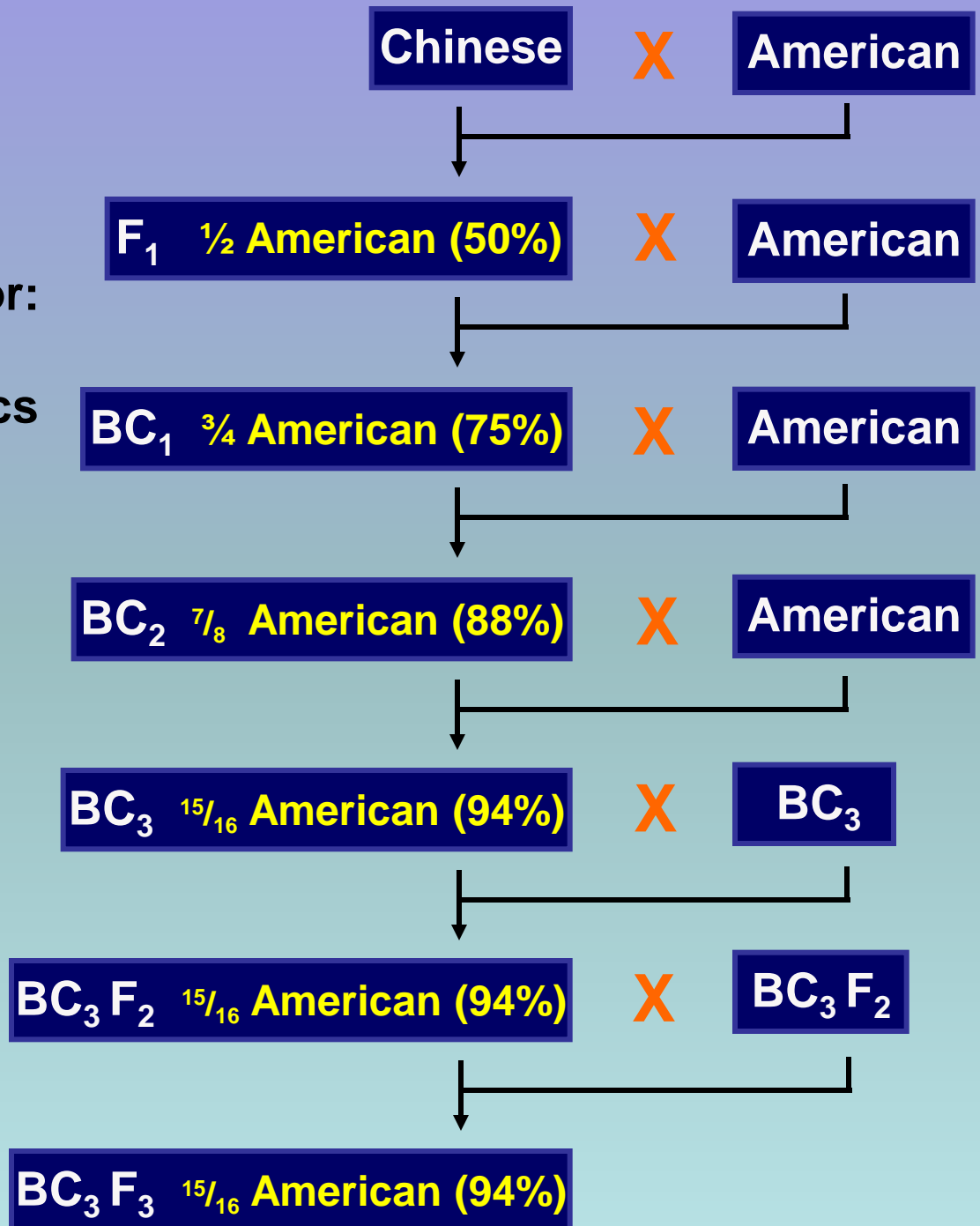
- Native birch species resistant to native bronze birch borer but exotic birch species are susceptible
- Native hemlocks (Carolina & Eastern) susceptible to exotic Hemlock wooly adelgid, exotic Chinese hemlock is resistant.
- First step in introgression is the production of interspecies hybrids. Resistant progeny are backcrossed to native species in order to carry only the resistance genes(s) into the native population
- Example: American Chestnut Foundation backcross breeding program

TACF's Backcross Breeding Program

Each generation select for:

- ❖ Blight resistance
- ❖ American characteristics

Final Product: goal is true-breeding American type with a high level of blight resistance



Development of Novel Ash Hybrids

to introgress resistance to Emerald Ash Borer into Native Ash Species

- **Two years of Controlled Pollinations**
 - 31 different species combinations
 - 190 pollination bags (multiple flowers per bag)
 - using 9 species as female, 10 species as male
 - using 1-5 trees per species
- **Putative Hybrid Seedlings**
 - 1619 total seed produced-much was heavily weeviled
 - Lots of trips up and down in bucket truck
 - 36 seedlings produced.
 - *F. chinensis* x *F. americana* – 2 seedlings**
 - *F. angustifolia* spp. *syriaca* x *F. mandshurica*
 - 10 seedlings (self-pollinations)
 - *F. angustifolia* spp. *syriaca* x *F. nigra* – 26 seedlings (self-pollinations)



This work was done in cooperation with Dawes Arboretum, Newark, OH

Barriers to Hybridization

- **Travel**

Sexually mature Asian ash species are not prevalent in the United States. Those that exist many times require extensive travel to carry out pollinations.

Accession, Replicate, and Establish at USFS Lab at Delaware OH

- **Phenology**

Coordinating pollen release with female flower periods of receptivity can be challenging. Timing of flower maturation in Asian species growing in the United States is not well documented and may differ from when it occurs in its native habitat.

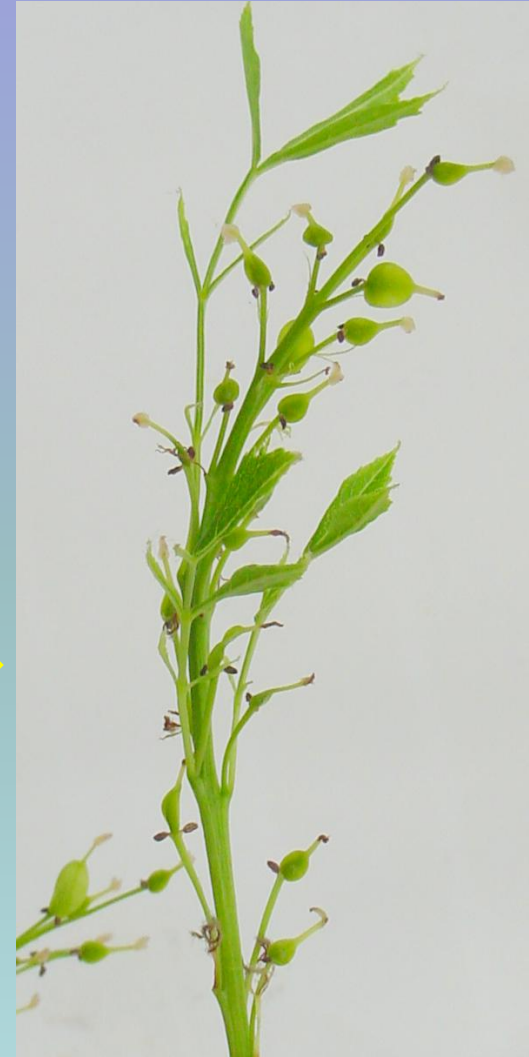
Pollinate potted, grafted ramets in the greenhouse

- **Different breeding systems between species:**

androdioecy, dioecy, polygamy, polygamodioecy, hermaphroditism
wind-pollination vs. insect pollination

Accession parents of both sexes where applicable

Grafted, containerized, flowering ash species:



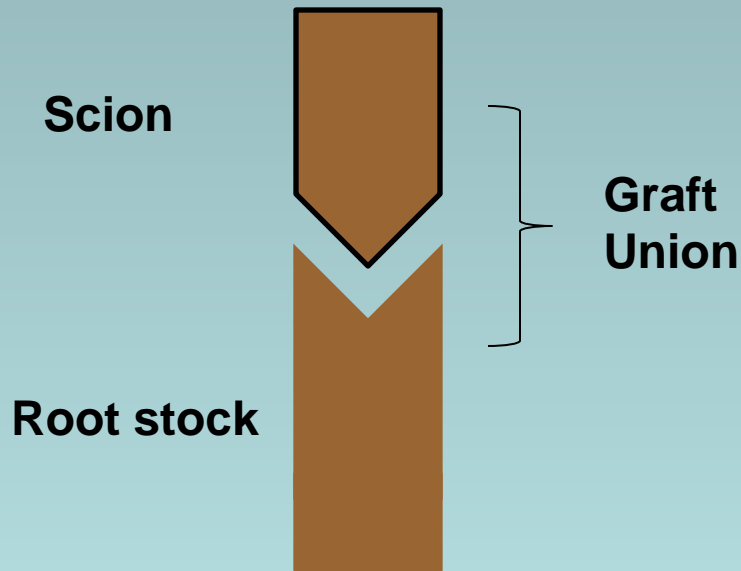
F. angustifolia spp. *syrica*

Ash Grafting

Top Grafting:

Cut scion and rootstock with topgrafting tool.

Used when scion is large and abundant. Can get grafted material to flower quickly.



Hot Callus Grafting

Scion: just above
freezing

Graft union: use heat coil
to keep around room temp

Rootstock: just above
freezing



Ash Grafting

Bud grafting (left) and growing grafts (right) of lingering ash at the US Forest Service Delaware Lab.



- Bud grafting effective when flowering/breeding is not primary objective or small amount of scion material available.
- Can produce a lot of material from a small amount of scion.

Exotic Ash Accessions/Potential Parents for Hybridizations:

(7 Asian species, 3 European species)

Species	Origin	Unique Accessions
<i>F. chinensis</i>	China/Korea	24
<i>F. excelsior</i>	Europe	3
<i>F. holotricha</i>	Balkans	2
<i>F. lanuginosa</i>	Japan	1
<i>F. mandshurica</i>	China, Korea, Russia, Japan	23
<i>F. ornus</i>	Europe	3
<i>F. sieboldiana</i>	Chin, Korea, Japan	3
<i>F. sogdiana</i>	China	3
<i>F. spathiana</i>	Japan	1
<i>F. syriaca</i>	Syria	1

Barriers to Hybridization

- **Genetic incompatibility**

Species differences in genomes can lead to lethal mutations, especially in case of interspecies hybridization between distantly related species.

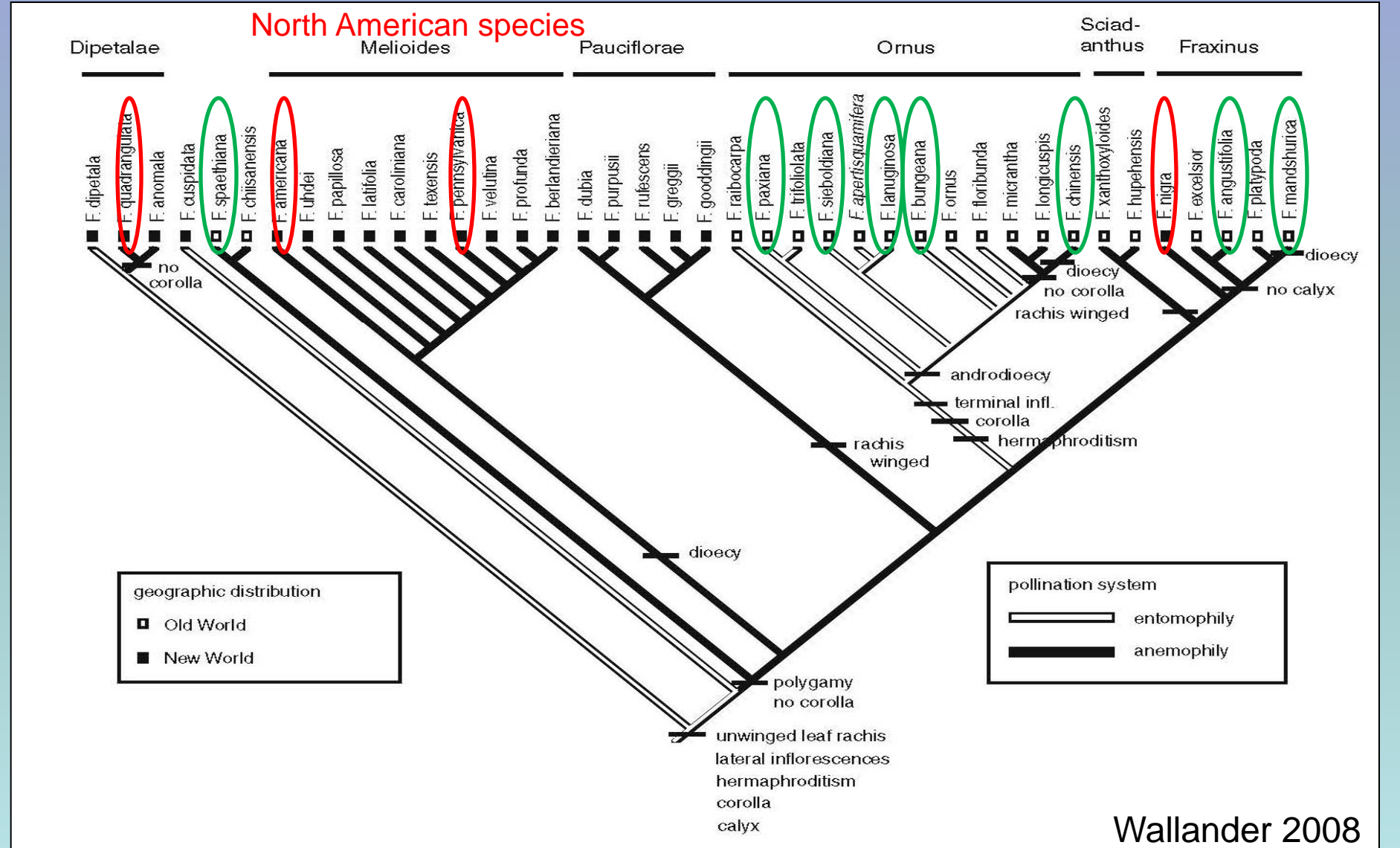
Try to breed trees that are closely related.

Genus Fraxinus

Minimize potential for genetic incompatibility by hybridizing species that are closely related phylogenetically (based on ITS sequence)

Asian species

North American species



Fraxinus spaethiana



University of Tokyo Botanical
Garden, Japan

Dawes Arboretum, Newark, OH

US Forest Service, NRS, Delaware, OH

New Accessions from China

NACPEC (North America-China Plant Exploration Consortium)

Seed Collection in China

Also new accessions of Asian species already in our collection

Kris Bachtell, Morton Arboreum

F. asperisquamifera

F. insularis

F. paxiana

F. stylosa

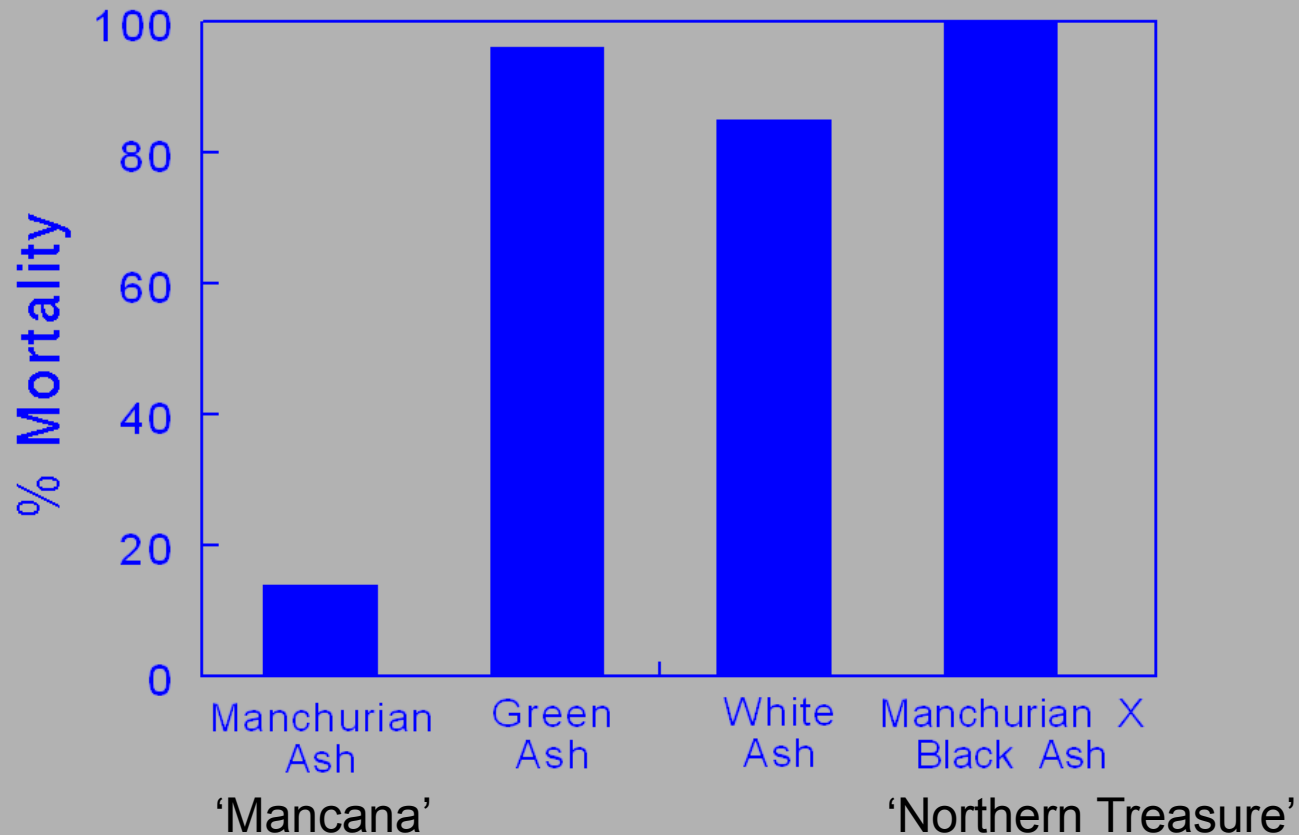


Successful Hybridization of North American With Asian Ash

- Dr. Wilbert Ronald Morden Research Station,
Agriculture Canada, 1969
- Goal: to create cold hardy tree
- Crossed *F. nigra* (female) with *F. mandshurica* (male)
- Released two hybrid cultivars, Northern Gem (US patent PP11,772P2) and Northern Treasure (US patent PP11,840P2) in 1997 (Dr. Campbell Davidson)
- Also released *F. mandshurica* cultivar 'Mancana'

Common Garden Study, Novi, MI

Rebek et al. 2008. *Environ Entomol* 37:242-246



- Need to look at more progeny to see segregation of resistance or resistance may be recessive trait requiring backcrossing.
- Need to test Northern Gem

Containerized Controlled Cross-Pollinations 2010

- Focus on combinations of *F. nigra*, *F. mandshurica*, Northern Treasure and Northern Gem---only known successful hybridization

F. nigra x *F. mandshurica* = Northern Gem, Northern Treasure

F. nigra female parents	F. mandshurica male parents
F. nigra-1	F. mandshurica-5
F. nigra-10	F. mandshurica-16
F. nigra-11	F. manshurica-23
F. nigra-5	


- 12 different combinations, all failed
- perfect flower, self-pollination?

Containerized Controlled Cross-Pollinations 2010

-Can *F. nigra* and *F. mandshurica* cross breed bidirectionally?

F. mandshurica x *F. nigra* = ?????

<i>F. mandshurica</i> female parents	<i>F. nigra</i> male parents
F. mandshurica-15	F. nigra-1
F. mandshurica-20	F. nigra-5*
F. mandshurica-1	
F. mandshurica-2	
F. mandshurica-3	



-10 different combinations

-3 cross combinations appear to be successful

F. nigra 5 x F. man15
x F. man 1
x F. man 3

Containerized Controlled Cross-Pollinations

F. mandshurica x *F. nigra*

2010



***F. mandshurica* female flower**



***F. nigra*, male flower**

Containerized Controlled Cross-Pollinations

F. mandshurica x *F. nigra*

2010



F. mandshurica x *F. nigra*
developing seed



F. mandshurica x *F. nigra*
seedling

Northern Treasure & Northern Gem Backcrosses and Intercrosses

- American Chestnut Foundation uses backcrossing of hybrid to American parent to increase the proportion of American traits in each subsequent generation.
- Intercrosses (cross between 2 progeny of same generation) used to stack multiple resistance genes (2 or 3 major resistance loci in chestnut).
- In ash, we can use these crosses to learn more about how resistance is inherited: how many genes, dominant, recessive, quantitative, etc.?

Northern Treasure & Northern Gem (*F. nigra* x *F. mandshurica*) Intercross

Female Parent	Male Parent
Northern Treasure	Northern Treasure
	Northern Gem

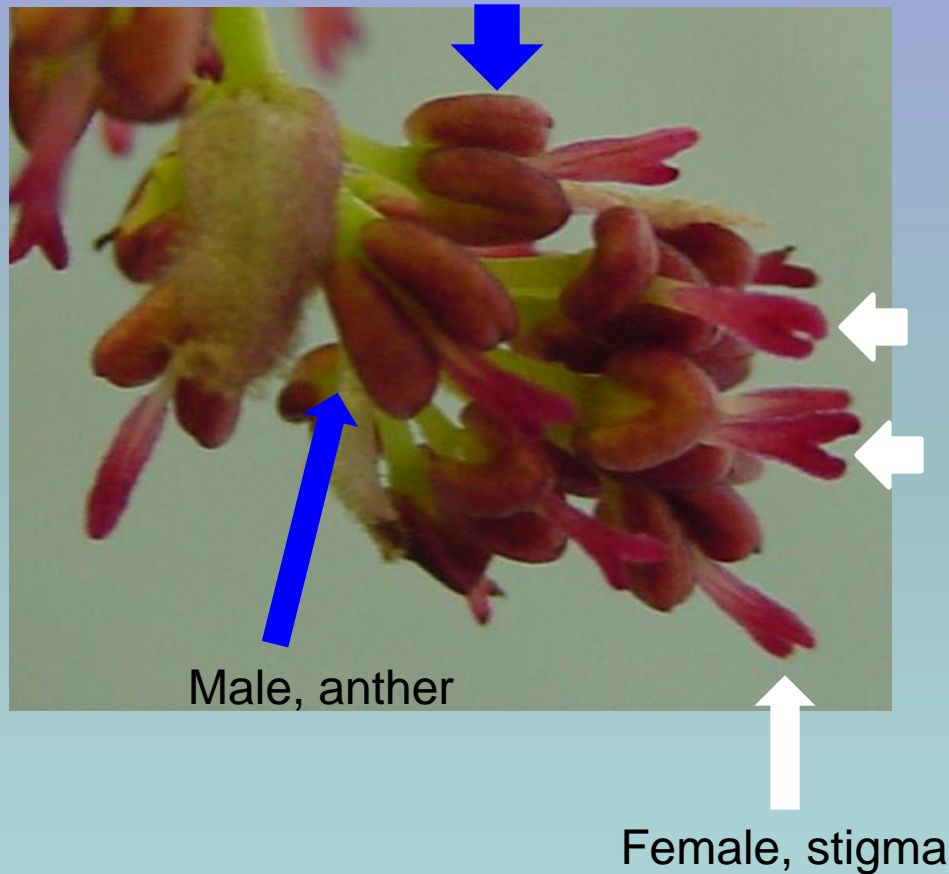
Backcross

Female Parent	Male Parent
Northern Treasure	F. mandshurica-5
	F. mandshurica-16
	F. mandshurica-23

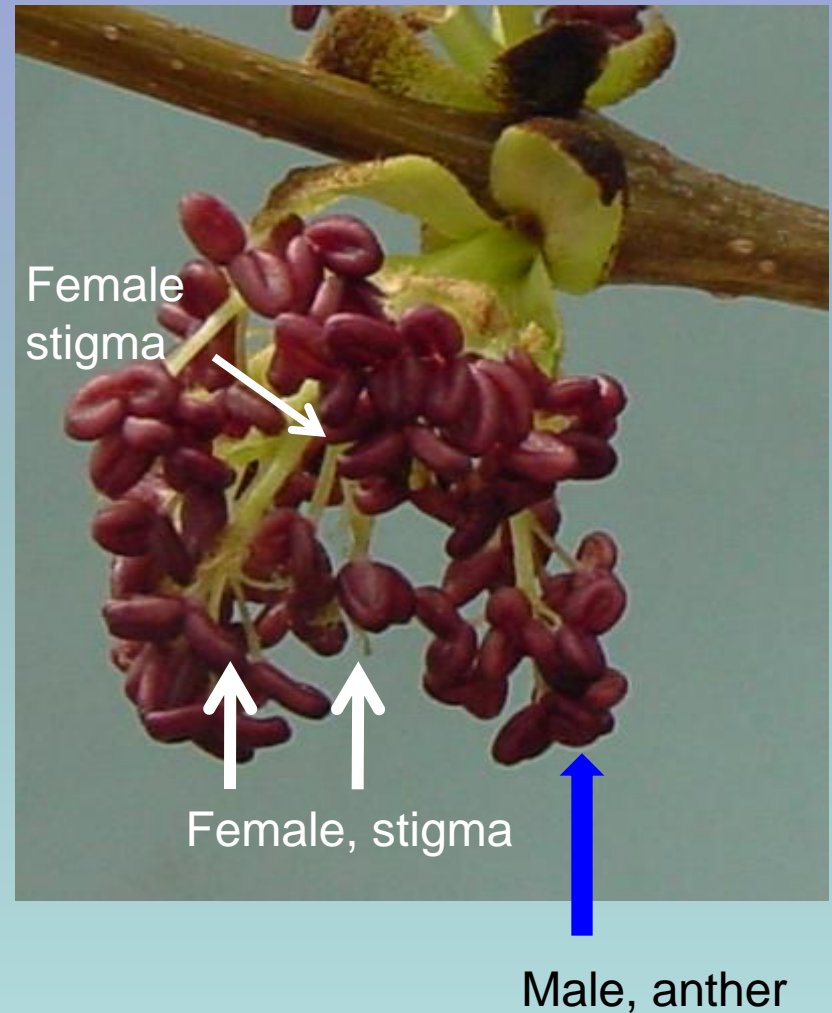
All crosses failed.

Northern Treasure donated for research purposes by Bailey's Nursery

F. quadrangulata perfect flower



Northern Gem perfect flower



The female flower structures on Northern Gem are very small... possibly nonfunctional-which is a desired feature of horticultural cultivars

Northern Treasure & Northern Gem (*F. nigra* x *F. mandshurica*) Backcrosses

Female Parent	Male Parent
F. mandshurica 15*	Northern Treasure
F. mandshurica 20*	Northern Gem
F. mandshurica 1	
F. mandshurica 2	
F. mandshurica 3	
F. nigra 1*	
F. nigra 5	
F. nigra 10	
F. nigra 11	

Out of 18 different cross combinations, 3 are currently setting seed.

Breeding Program to Produce EAB Resistant Black Ash?



Seed developing from a *F. mandshurica* x Northern Gem cross

- hybrids Northern Gem and Northern Treasure can be backcrossed to parent species, but only as pollen donor.
- if resistant hybrid produced, this resistance potentially can be “moved” into black ash through a long-term breeding program
- based on very preliminary results!

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